REMARKS

Claims 20 and 22-23 are all the claims pending in the application.

The claims have been amended to more clearly state that the antibodies encompassed within the scope of the claims are those that bind to an epitope within the amino acid sequence of SEQ ID NO:1 or a sequence with 70% homology to SEQ ID NO:1 (claim 20), or an epitope of a protein expressed by a cell transformed with a vector encoding SEQ ID NO:2 or SEQ ID NO:3 (claims 22 and 23, respectively). While the present specification fully describes the production of anti-LY6H antibodies (page 31, line 25, through page 35, line 6), it does not explicitly use the term 'epitope' to describe the portion of the LY6H protein that a specific anti-LY6H antibody binds. However, Applicants assert that the skilled artisan would implicitly understand that an antibody recognizes and binds a specific 'epitope' of a protein, and not the protein in its entirety. As such, the amendments of the claims and the inclusion of the term 'epitope' have not introduced any new matter.

Entry of the amendment is respectfully solicited.

I. Formal Matters

Applicants note that the Examiner has not yet acknowledged Applicants' claim to priority under 35 U.S.C. §119(a)-(d), asserted in the application papers filed March 16, 2001. Applicants thus now request that the Examiner acknowledge receipt of the priority documents in this application, and perfection of Applicants' claim to priority.

II. Rejection of Claims Under 35 U.S.C. §101

At paragraph 5 of the Office Action, claims 20, 22 and 23 stand rejected under 35 U.S.C. §101, as being drawn to an invention with no apparent or disclosed specific and substantial credible utility.

The Examiner refers to the reasons of record in the Office Action dated December 13, 2001, briefly summarized as that the instant application has provided a description of an antibody to a protein encoded by an isolated DNA. However, the Examiner alleges, the instant application does not disclose the biological role of this protein or its significance.

The Examiner responds to the statements made in the Horie Declaration by stating that the Declaration is insufficient to overcome the rejection because it does not establish the biological significance of the LY6H protein.

In response, Applicants enclose herewith a second Declaration Under 37 C.F.R. §1.132 by Masato Horie, further demonstrating that, in contrast to the Examiner's position, the present specification discloses a biological role for the LY6H protein of the present application, and antibodies that recognize and bind this protein. The declaration establishes that the LY6H protein shows the brain memory-forming activity claimed in the present application.

For example, as revealed by the data from Experiment 1 discussed in the declaration, the mnemonic action, evaluated according to the method described in the specification (page 39, lines 4 to 7) of the LY6H knockout mice (having lost LY6H expression in basolateral amygdala), is significantly reduced compared with that of the control wild-type mice (expressing LY6H).

Furthermore, the results obtained from Experiment 2 indicate that the LY6H knockout mice show reduced memory consolidation (failing to memorize the electrical shock), as compared to wild-type mice.

Thus, the LY6H protein clearly plays an important role in memory formation of the brain. More specifically, the LY6H protein has a "brain memory-forming activity" as one of its functions.

The clear and credible utility of the antibody is evidenced on page 34, line 19 to page 35, line 4, of the specification. That is, "the antibody of the present invention can be used for the purification of LY6H protein by immunological techniques and utilized in the screening for agonists or antagonists of LY6H protein."

Thus, the declaration establishes the biological significance of the LY6H protein and a credible utility of the antibody of the invention.

In view of these comments, and the second declaration of Dr. Horie, Applicants respectfully request reconsideration and withdrawal of this rejection.

III. Rejection of Claims Under 35 U.S.C. §112

A. At paragraph 6 of the Office Action, the rejection of claims 20, 22 and 23 under 35 U.S.C. §112, first paragraph, as being non-enabled, has been maintained.

The Examiner generally asserts that as no utility has been established for the anti-LY6H antibodies of the present invention, the skilled artisan would not know how to use the antibodies.

In response, Applicants assert that a skilled artisan would generally understand how to make and use antibodies for a number of purposes. Further, the specification provides specific

examples of uses for anti-LY6H protein antibodies (*see, e.g.*, page 34, line 19 through page 35, line 4).

As a utility for the LY6H protein, and antibodies that recognize and bind it, has been established, and the skilled artisan would be enabled to make and use the antibodies that bind this protein, Applicants respectfully request reconsideration and withdrawal of this rejection.

B. At paragraph 7 of the Office Action, claims 20, 22 and 23 are rejected under 35 U.S.C. §112, first paragraph, as being non-enabled.

The Examiner asserts that the claims are not enabled due to the recitation "comprising an amino acid sequence of SEQ ID NO: 1" in claim 20, and "comprising the nucleic acid sequence of SEQ ID NO: 2 [or SEQ ID NO: 3]" in claims 22 and 23. The Examiner states that the cited claims encompass an antibody which binds to an epitope that is not contained within SEQ ID NO: 1. For example, the Examiner states that unrelated epitopes (e.g., the FLAG tag) could be included in the polypeptide "comprising" the amino acid sequence as set forth in SEQ ID NO: 1. Thus, the claims essentially encompass an antibody which can bind to any polypeptide or protein.

The Examiner goes on to state that the specification does not provide a written description or the guidance needed to produce an antibody which binds to any epitope other than an epitope which is contained within SEQ ID NO: 1.

In response, Applicants include herewith amendments to the claims such that the antibodies recited therein are limited to only those antibodies that recognize and bind the LY6H protein (i.e., LY6H epitopes). Further, given the disclosure of the amino acid sequence of the

LY6H protein in the specification, Applicants assert that a skilled artisan would be enabled to make and use the antibodies of the present invention.

Given the amendments to the claims, Applicants further assert that adequate written description of the antibodies encompassed within the scope of the claims is provided in the application as filed.

In view of the amendments to the claims and the comments above, Applicants assert that the claims are fully enabled and meet the written description requirement, and therefore respectfully request reconsideration and withdrawal of this rejection.

C. At paragraph 8 of the Office Action, claims 22 and 23 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite.

The Examiner states that the use of the phrase "an expression product expressed by a host cell" renders the cited claims indefinite because transformed host cells produce more than one expression product.

In response, Applicants note that the claims have been amended to more clearly recite Applicants' invention, namely antibodies that bind epitopes of the LY6H protein.

In view of the amendments to the claims, Applicants assert that the claims are definite as stated, and respectfully request reconsideration and withdrawal of this rejection.

IV. Rejection of Claims Under 35 U.S.C. §102

At paragraph 9 of the Office Action, claims 20, 22 and 23 are rejected under 35 U.S.C. §102(b) as being anticipated by Hopp et al. (U.S. Patent No. 5,011,912).

The Examiner states that because the claims encompass an antibody which binds to any antigenic peptide, they are anticipated by Hopp et al. which teaches the FLAG epitope.

In reply, Applicants again note that the claims have been amended to limit the scope of the claims to those antibodies that bind epitopes of the LY6H protein.

As Hopp et al. does not teach or suggest anti-LY6H antibodies, this reference does not anticipate the pending claims, as amended herein.

In view of these comments and the amendments to the claims, Applicants respectfully request reconsideration and withdrawal of this rejection.

V. Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

Drew Hissong
Registration No. 44,765

SUGHRUE MION, PLLC
Telephone: (202) 293-7060
Facsimile: (202) 293-7860

WASHINGTON OFFICE

23373
PATENT TRADEMARK OFFICE

Date: November 26, 2002

APPENDIX
VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

The claims are amended as follows:

20. (Twice amended) An antibody which binds specifically to a protein comprising an amino acid sequence of SEQ ID NO: 1, or an amino acid sequence which is ~~with~~ at least 70% homologous ~~homology~~ to the amino acid sequence of SEQ ID NO: 1, wherein the antibody binds to an epitope within the amino acid sequence of SEQ ID NO: 1, or binds to an epitope within the amino acid sequence which is at least 70% homologous to the amino acid sequence of SEQ ID NO: 1, and the protein exhibits at least one physiological activity selected from the group consisting of neuronal survival-supporting activity, nerve elongating activity, nerve regenerating activity, neuroglia~~neoroglia~~-activating activity and brain memory-forming activity.

22. (Amended) An antibody which binds specifically to an epitope encoded by a nucleic acid sequence represented by SEQ ID NO: 2, wherein said epitope is a portion of an expression product of a host cell and wherein said expression product is encoded by an expression vector transfected into said host cell and said expression vector comprises ~~expression product expressed by a host cell comprising an expression vector comprising~~ a DNA molecule comprising the nucleotide sequence of SEQ ID NO: 2, operably linked to a promoter.

23. (Twice amended) An antibody which binds specifically to an epitope encoded by a nucleic acid sequence represented by SEQ ID NO: 3, wherein said epitope is a portion of an expression product of a host cell and wherein said expression product is encoded by an expression vector transfected into said host cell and said expression vector comprises ~~expression~~

~~product expressed by a host cell comprising an expression vector comprising~~ a DNA molecule comprising the nucleotide sequence of SEQ ID NO: 3, operably linked to a promoter.

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Masato HORIE, et al.

Appln. No.: 09/787,360

Confirmation No.: 7873

Filed: March 16, 2001

For: LY6H GENE

Docket No: Q63396

Group Art Unit: 1646

Examiner: Chernyshev, O.

#12
B.GJ
12/11/02

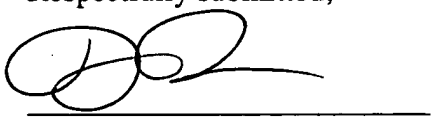
SUBMISSION OF EXECUTED DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith is an executed Declaration Under 37 C.F.R. §1.132, signed by Masato HORIE.

Respectfully submitted,



Drew Hissong
Registration No. 44,765

SUGHRUE MION, PLLC
2100 Pennsylvania Avenue, N.W.
Washington, D.C. 20037-3213
Telephone: (202) 293-7060
Facsimile: (202) 293-7860

Date: November 26, 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Masato HORIE et al.

Appln. No. 09/787,360 Group Art Unit: 1646

Filed: March 16, 2001 Examiner: Olga N. Chernyshev

For: LY6H GENE

DECLARATION

Honorable Commissioner of Patents and Trademarks

Washington, D.C. 20231

Sir:

I, Masato HORIE, senior researcher, Second Institute of New Drug Discovery, Otsuka Pharmaceutical Co., Ltd., hereby declare that:

1) I am one of the inventors of the instant invention,

2) I graduated from Hokkaido University in 1985 and have been involved in the LY6H project since 1997, and

3) this document is to provide evidence to show the biological role of the LY6H protein and the significance thereof.

I. Introduction

A "brain memory-forming activity" is set forth in the present specification as one of the physiological activities exhibited by the LY6H protein.

The following experiments were carried out to show more clearly that the LY6H protein is closely related to the brain memory-forming activity and that it is, therefore, necessary for memory formation, using laboratory animals showing reduced LY6H expression in the basolateral amygdala, i.e., LY6H knockout mice.

II. Experiment 1

The Morris water-maze test (Nature 297: 681 to 683, 1982, attached herewith) was used in Experiment 1 as described below in more detail. This test is also recited on page 39, lines 4 to 7, of the present specification.

A "hidden platform" version of the Morris water-maze test was conducted to assess spatial learning ability using adult male mice. The circlular tank (36 cm in height x 90 cm in diameter) of the apparatus was filled with water maintained at 21 $\pm$ 1°C and made opaque with nontoxic black paint. The surface of the platform (11 cm in diameter) was 5 mm below the water surface. Latency for reaching the platform was recorded for eight successive days.

Fig. 1 shows the results. It is understood from Fig. 1 that the spatial memory of the LY6H knockout mice is significantly reduced compared with that of the control wild-type mice.

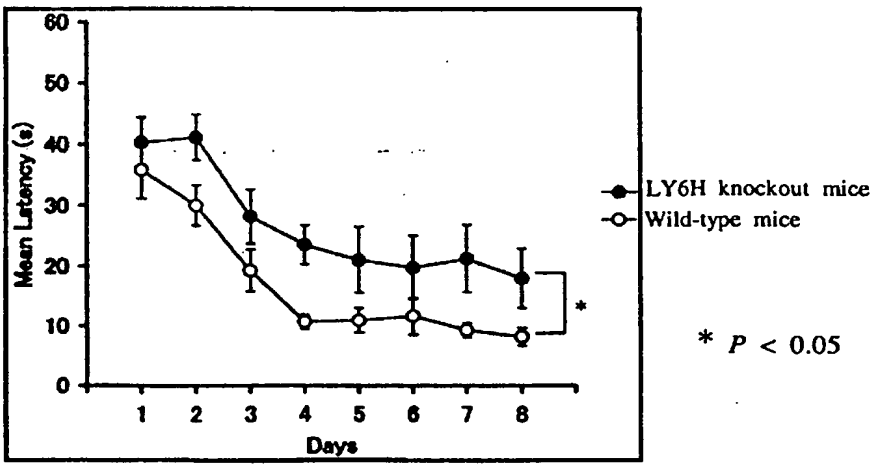


Fig. 1 Impaired spatial memory in LY6H knockout mice.

The latency (sec) for reaching the platform was measured for the LY6H knockout (solid circles, n = 11) and the wild-type (solid squares, n = 11) mice. Each point represents the mean ± SEM.

III. Experiment 2

A passive avoidance test was also used for proving that the LY6H protein is directly related to brain memory-forming activity and is necessary for memory formation.

A step-through type passive avoidance apparatus was used for this passive avoidance test. This apparatus

consisted of two compartments, one light (10 x 10 x 20 cm) and one dark (10 x 10 x 20 cm), with a grid floor. A guillotine door separated the two compartments.

In the acquisition trial, mice were individually placed in the light compartment. Five seconds later, the door to the dark compartment was opened. When the mouse moved into the dark compartment, the guillotine door was closed, and 10 and 18 seconds later a scrambled electrical shock (100 V, 3 sec) was delivered through the floor grids by a shock generator. The mouse was then removed from the apparatus and returned to its home cage. Twenty-four and forty-eight hours later, each mouse was placed in the light compartment and the step-through latency (sec) for entering the dark compartment was measured in the retention trial.

The results are shown in Fig. 2. As understood from Fig. 2, the data obtained at Days 1 and 2 reveal that the LY6H knockout mice failed to memorize the electrical shock presented at Day 0 ($P > 0.05$ against Day 0), while the control wild-type mice successfully acquired the memory of the shock ($P < 0.01$ against Day 0).

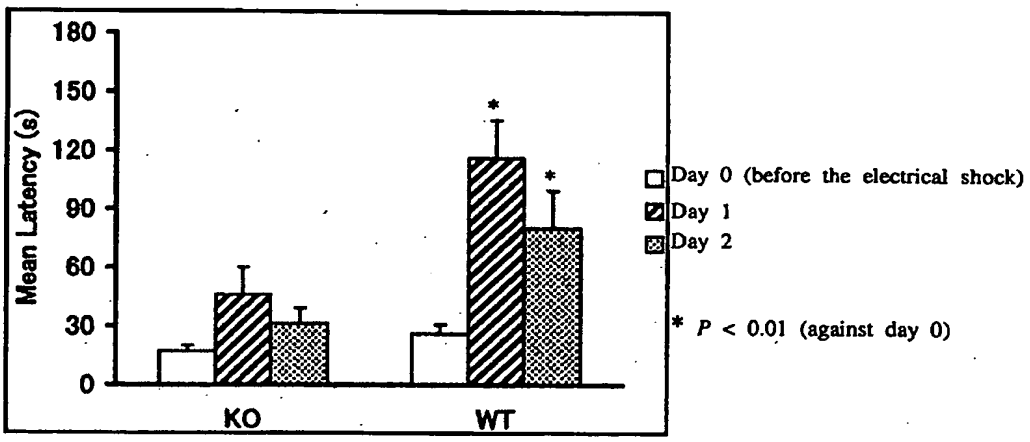


Fig. 2 Memory deficits of LY6H knockout mice in the passive avoidance task.

The latency for entering the dark compartment was measured for the LY6H knockout (KO, n = 11) and wild-type (WT, n = 11) mice. Each point represents the mean ± SEM.

IV. Conclusion

These findings show that a loss of LY6H expression results in a dramatic impairment of memory consolidation. Since the amygdala plays a crucial role in consolidating memory (for review, see Trends Neurosci. 25: 456-461, 2002, attached herewith), the reduction of LY6H expression in the amygdala of Alzheimer's patients and our data on LY6H knockout mice illuminate the biological significance of LY6H and its relation to this devastating disease. Thus, the reduction of LY6H expression in amygdala can be

expected to contribute to the impairment of memory consolidation in Alzheimer's patients.

I, the undersigned, declare that, to the extent of my knowledge, all statements made herein are true and that all statements made based on information and beliefs are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Nov. 19, 2002

Masato Horie

Masato Horie, D.V.M., Ph.D.

BEST AVAILABLE IMAGES

Defective images within this document are accurate representation of The original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

will thus be very small, therefore the stimulation of the lateral line by the mechanism discussed here will be of litle consequence. This means that lateral line systems should be disturbed little by most of the background noises in the sea[4,6,12].

We thank Mr C. R. Griffiths for generous help. J.A.B.G. is a member of the external scientific staff of the MRC. The Association is grant-aided by the NERC.

Received 17 March; accepted 26 April 1982.

1. Harris, G. G. & Van Bergeijk, W. A. *J. acoust. Soc. Am.* **34**, 1831–1841 (1962).
2. Dijkgraaf, S. *Biol. Rev.* **38**, 51–105 (1963).
3. Kuiper, J. W. *Lateral Line Detectors* (ed. Cahn, P.) 105–121 (Indiana University Press, 1967).
4. Sand, O. *Hearing and Sound Communication in Fishes* (eds Tavolga, W. N., Popper, A. N. & Fay, R. R.) 459–480 (Springer, New York, 1981).
5. Sand, A. *Proc. R. Soc.* B123, 472–495 (1937).
6. Denton, E. J., Gray, J. A. B. & Blaxter, J. H. S. *J. mar. biol. Ass. U.K.* **59**, 27–47 (1979).
7. Blaxter, J. H. S., Denton, E. J. & Gray, J. A. B. *Hearing and Sound Communication in Fishes* (eds Tavolga, W. N., Popper, A. N. & Fay, R. R.) 39–59 (Springer, New York, 1981).
8. Harris, G. G. *Marine Bioacoustics* (ed. Tavolga, W. N.) 233–247 (Pergamon, Oxford, 1964).
9. Enger, D. S. *Comp. Biochem. Physiol.* **22**, 527–538 (1967).
10. Hawkins, A. D. & Horner, K. *Sound Communication in Fishes* (eds Tavolga, W. N., Popper, A. N. & Fay, R. R.) 311–328 (Springer, New York, 1981).
11. Partridge, B. L. & Pitcher, T. J. *J. comp. Physiol.* **135**, 315–325 (1980).
12. Urick, R. J. *Principles of Underwater Sound* (McGraw-Hill, New York, 1967).

Place navigation impaired in rats with hippocampal lesions

R. G. M. Morris*, P. Garrud*, J. N. P. Rawlins† & J. O'Keefe‡

* Psychological Laboratory, University of St Andrews, St Andrews, Fife KY16 9JU, UK
† Department of Experimental Psychology, University of Oxford, Oxford OX1 2JD, UK
‡ MRC Cerebral Mechanisms Unit, University College, University of London, London WC1E 6BT, UK

Electrophysiological studies have shown that single cells in the hippocampus respond during spatial learning and exploration[1–4], some firing only when animals enter specific and restricted areas of a familiar environment. Deficits in spatial learning and memory are found after lesions of the hippocampus and its extrinsic fibre connections[5,6] following damage to the medial septal nucleus which successfully disrupts the hippocampal theta rhythm[7], and in senescent rats which also show a correlated reduction in synaptic enhancement on the perforant path input to the hippocampus[8]. We now report, using a novel behavioural procedure requiring search for a hidden goal, that, in addition to a spatial discrimination impairment, total hippocampal lesions also cause a profound and lasting place-navigational impairment that can be dissociated from correlated mot r, motivational and reinforcement aspects of the procedure.

If rats are placed in a large circular pool of opaque water, they will quickly learn to escape by finding and climbing on to a small platform hidden beneath the water surface, provided it remains in a fixed location over a series of trials[9]. They cannot learn to find it when its position varies randomly from trial to trial. Although they can never see, hear or smell the platform, rats require only a few trials in order to learn to swim directly towards it, using the shortest route, even from a novel starting place. That is, the rats learn not only to recognize the vicinity of the safe place when they reach it, but also to swim towards it from a distance despite the absence of cues from the platform itself. The deleterious effects of cue–response separation apparent in visual discrimination[10,11] do not, in this case, prevent extremely rapid learning. By comparing the performance of normal and brain-lesioned animals in these conditions with that sh wn when a fixed but visible platform was used, we have examined the role of the hippocampus in simple navigation.

Female Lister rats ($n = 31$) were subjected t the following procedures: total hippocampal lesions ($n = 10$), superficial cortical lesions ($n = 13$), sham surgery ($n = 4$) or no surgery ($n = 4$). The rats were placed, under pentobarbitone anaesthesia, into a special adjustable head-holder[12]. Animals in the hippocampal lesion group had holes drilled in their skulls, and a small amount of neocortex overlying the hippocampus, and the entire dorsal and ventral hippocampus were removed by aspiration. Operated control animals had comparable lesions in the neocortex but showed no hippocampal damage. Sham-operated control animals had burr holes drilled in their skulls but suffered no brain damage. On completion of the behavioural procedures, conventional histological techniques (40 μm, gelatine-embedded sections stained with cresyl violet and solochrome cyanide) were used to verify the lesions. All rats of the hippocampal-lesion group were found to have total or near total destruction of the dorsal and ventral hippocampus, with minimal damage to adjacent structures (comparable lesions are reported in ref. 13). Analysis of the behavioural data showed no differences between the sham-operated and unoperated control groups, which were therefore combined, giving final group sizes of 10 (hippocampal), 13 (cortical) and 8 (control).

On day 1, the rats were placed in a pool of water (1.32 m diameter, 53 l at 26 ± 1 °C) and allowed to swim freely for 1 min with no opportunity for escape. On day 2, a platform was hidden in one of four locations in the middle of each cardinal quadrant (SW, NW, NE and SE), 0.33 m from the side walls. Different locations were used for different rats. The platform, made of clear perspex, was hidden by adding 2.3 l of milk to the water and arranging for its top surface, 8 cm in diameter, to be 1 cm below the water level. A second platform, 2 cm taller and protruding visibly out of the water, was used at a later stage of training; its top surface was indented such that it contained within its circumference a 1 cm layer of water. Thus, the reinforcement afforded by escape on to the two platforms was equated. The rat's task throughout the training procedures, which continued for 8 days, was to find and escape on to the platforms. Only one platform was used at a given stage of training, and it was always in a fixed position in the pool on a given day. Thus the two tasks, which we shall call place-navigation and cue-navigation, respectively, involved the same motor movements (swimming), motivation and reinforcement (escape from water), but differed specifically and uniquely with respect to whether or not the rat was required to learn the platform's position in relation to the varied distal room cues.

All rats swam effectively using the characteristic adult swimming posture[14]. The times taken to escape from the water during the three successive phases of the experiment are shown in Fig. 1. The normal and cortical-lesion groups learned to escape rapidly from the water with stable terminal acquisition latencies of < 8 s. The hippocampal-lesion group showed a highly significant impairment in the place-navigation task (trials 1–28) when the hidden platform was used. However, this impairment declined dramatically and disappeared when the visible platform was used (trials 30–41), this platform having been placed diagonally opposite to the earlier training location (that is, NW for a rat trained previously to find the hidden platform at SE). The place-navigation impairment reappeared when training was continued with the hidden platform (trials 43–50) even though it remained in the same position that the visible platform had occupied in the preceding phase of training.

Detailed analysis of the behavioural performance of each group and the results of two transfer tests provide new insights into the nature and magnitude of the deficit after hippocampal lesions. First, the hippocampal-lesion animals did improve during training but never escaped faster than normal animals searching for a hidden platform that was moved around randomly from place to place on successive trials (see Fig. 1, horizontal broken line, taken from ref. 9). Second, analysis of the paths taken by all rats on trial 28, transcribed from videotape recordings, showed that the hippocampal-lesi n animals took longer and more circuitous routes to find the hidden platform

0028–0836/82/250681—03$01.00

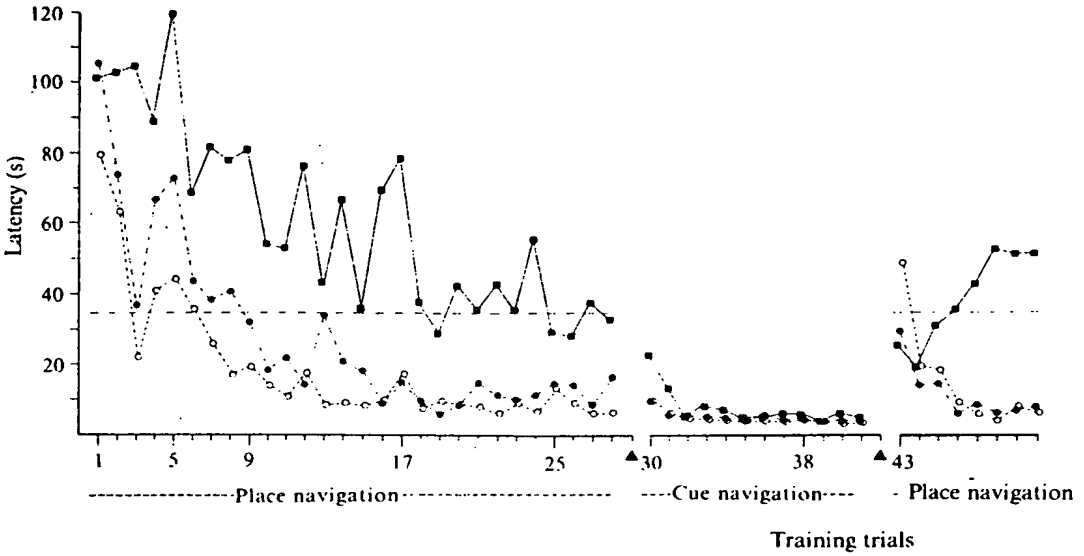


Fig. 1 Mean latency of escape (s) for the 50 trials of the experiment. ■, Hippocampal lesion; ●, cortical lesion; ○, control;. The trial number of the first of each daily set of trials is shown on the abscissa. The two transfer tests are indicated by solid triangles. To avoid problems of heterogeneity of variance, the successive phases of the experiment were analysed separately. The horizontal broken line (trials 1–28 and 43–50) at 34.5 s corresponds to the best performance shown by a group of normal rats trained to search in 20 trials for a hidden platform that was moved randomly from one place to another over successive trials (data taken from ref. 9). Place navigation (trials 1–28): unweighted means (unequal n) analysis of variance revealed significant effects of group ($F = 23.7$, d.f. = 2/28; $P < 0.0001$), trials ($F = 17.8$, d.f. = 23/667; $P < 0.0001$) and lesion × trials ($F = 1.5$, d.f. = 54/756; $P < 0.02$). Subsequent orthogonal comparisons showed that the deficit was restricted to the hippocampal-lesion rats (hippocampal versus cortical + control, $P < 0.0001$; cortical versus control, $P > 0.10$). Cue navigation (trials 30–41): terminal escape latencies (trial 41) were 5.0, 3.3 and 2.8 s for the hippocampal-lesion, cortical-lesion and control groups respectively, corresponding to declines relative to trial 28 of 45.9, 7.9 and 3.7 s). Analysis of variance of all 12 trials revealed a small residual impairment in the hippocampal-lesion groups ($F = 5.4$, d.f. = 2/28; $P < 0.02$). Return to place navigation (trials 43–50): analysis of variance showed a highly significant effect of groups ($F = 12.2$, d.f. = 2/28; $P < 0.0002$) and a lesion × trials interaction ($F = 3.6$, d.f. = 14/196; $P < 0.0001$). The apparent gradual impairment of performance in the hippocampal-lesion group was caused by a slowing of swimming speed over trials as the core temperature of the rats fell slightly (from 37 °C to ~35 °C).

(Fig. 2). The directional heading of the hippocampal-lesion rats when they set off from their starting position on trial 28 was no more likely to be towards the platform than in any other possible direction. These results imply that hippocampal-lesion rats can learn some sort of escape strategy (for example, that escape is possible) but are substantially poorer at learning where the hidden platform is located and, unlike normal and cortical-lesion animals, will never learn to swim towards it from a distance.

The magnitude of this place-navigational deficit was assessed in two separate transfer tests conducted on trials 29 and 42, immediately after the four daily trials of days 6 and 8. For transfer test A, the hidden platform was first removed from the apparatus, then the rats were placed in the pool for 60 s with no opportunity for escape, and their movements observed. The results were striking. Control and cortical-lesion rats swam to and persistently across the former platform location whereas the hippocampal-lesion rats did not. The hippocampal-lesion rats did not merely swim around the side walls. To demonstrate this, annuli were marked on the video screen indicating the exact surface area and former positions of the platforms in each of the four cardinal quadrants. The total number of annuli which an individual rat passed through during the 60-s test was 7.6, 6.8 and 8.6 for the hippocampal-lesion, cortical-lesion and control groups, respectively ($F < 1$). The groups were distinguished by which annuli they passed through: an individual hippocampal-lesion rat was no more likely to pass through the annulus marking the platform position used during training than one in any other quadrant (Fig. 3a). We observed no tendency on the part of the hippocampal-lesion rats to remain in the vicinity of the training annulus once they had eventually reached it (compare with ref. 7). Thus the deficit produced by hipp campal lesions was total. Furthermore, with respect to the lack of spatial bias revealed in the annulus measure, the deficit was apparent in all 10 rats of the experimental group.

Our interpretation of these findings is that, whatever their ther effects[15–19], hippocampal lesions do cause a profound and lasting place-navigational impairment. It could be argued, how v r, that while matched for motor requirements, motivation and reinforcement, the place- and cue-navigational tasks are not matched for task complexity. Perhaps hippocampal-lesi n animals perform poorly on the spatial task because it is complex (albeit a task learned by normal animals in less than 10 trials), and perform better on the visible platform task because it is easier, rather than because the spatial component is then redundant. If this is the basis of the dissociation of effects in the two tasks, then at least some spatial bias should be shown by some of the hippocampal-lesion animals in a transfer test conducted after training on the ostensibly easier visible platform task. Transfer test B, conducted immediately after trial 41 on day 8, examined this possibility. In trial 41 itself, there was no significant difference in the latency, path-length or directional ity of escape behaviour across groups ($F_s < 1$), all animals escaping rapidly by means of short, direct paths to

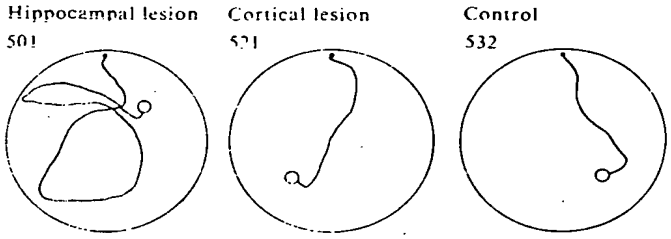


Fig. 2 The actual path of the median rat (defined in terms of path length) in each group on trial 28 just before the first transfer test. The rats were observed using a video camera placed above the pool. One experimenter (P.G.) sat concealed in one corner of the room and monitored the rat's movement on a VTR recorder. The second experimenter (R.M.) removed each rat from its home cage in an adjacent room and placed it in the pool. The pool was open to the room which included a door, a window, and brightly and darkly lit walls. The paths taken by the rats in escaping were transcribed from the videotape and measured. Path lengths: the hippocampal-lesion rats took 4.66 ± 0.86 m to reach the platform, whereas the cortical-lesion and control rats took 2.35 ± 0.98 and 1.20 ± 0.34 m, respectively. Analysis of variance showed that these path lengths differed significantly ($F = 4.23$, d.f. = 2/28; $P < 0.025$). Subsequent orthogonal comparisons showed that the hippocampal-lesion group took significantly longer paths than both the cortical-lesion and control groups ($P < 0.001$), which in turn did not differ significantly from each other ($P > 0.10$). Directionality: the accuracy of the approach to the platform was analysed as follows. We measured the angle subtending a tangent to the rat's path at a point 0.5 m from its starting position, and a line intersecting this point and the centre of the platform. This angle was 83 ± 18° from the correct direction for the hippocampal-lesion group, whereas for the cortical-lesion and control groups, the angle was 34 ± 13° and 38 ± 17°, respectively (Kruskal–Wallis, $H = 5.87$, d.f. = 2; $P < 0.025$, one-tailed).

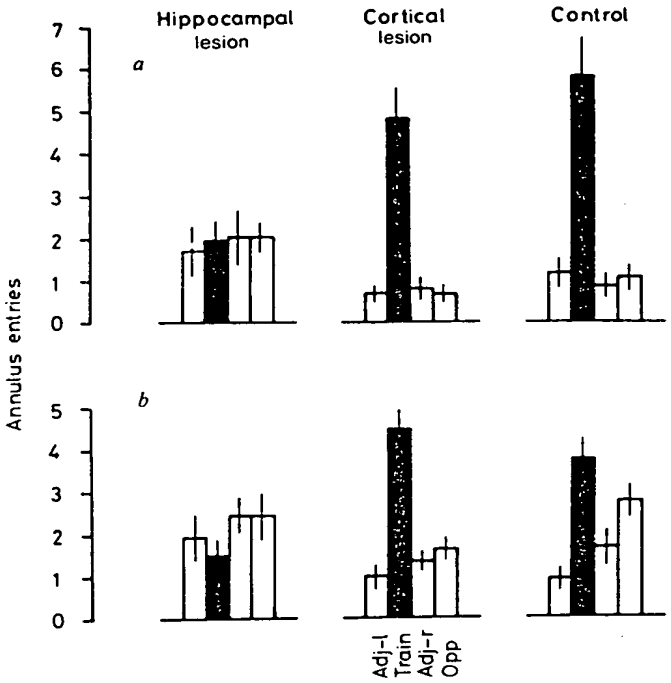


Fig. 3 Mean crossings of each of the annuli (± 1 s.e.) marking the former platform positions during *a*, transfer test A (after place-navigation training) and *b* transfer test B (after cue-navigation training). The data have been categorized for each animal into crossings of the training location (Train), and of the annulus in the adjacent quadrant to the left (Adj-l; viewed from above), the adjacent right (Adj-r), and opposite quadrant (Opp). Note that the hippocampal-lesion rats were no more likely to pass through the annulus marking the training position than any other, in both transfer tests. Analyses of variance showed a highly significant groups x position effect in both transfer test A ($F = 10.1$, d.f. = 6/84; $P < 0.0001$) and transfer test B ($F = 9.5$, d.f. = 6/84; $P < 0.0001$).

the visible platform. However, in the transfer test conducted 30 s later, only the control and cortical lesion groups searched in the vicinity of the now absent but previously visible platform (Fig. 3*b*). All 10 animals in the hippocampal-lesion group showed no spatial bias. Thus even if the improvement by the hippocampal-lesion group in the visible platform phase of training was due to the simplicity of the task, this improvement was not accompanied by any spatial learning.

The procedures used here provide a new approach to analysing the brain mechanisms of spatial localization. The results show that hippocampal lesions cause a profound and lasting impairment in place-navigation and question that aspect of the working-memory hypothesis[17] which asserts that spatial reference memory is unaffected by septo-hippocampal damage. Reference memory has been defined as those aspects of a learning procedure in which learned information may be used in every trial of training rather than for just a single trial. The present procedure using a fixed platform position for 28 trials, followed by different fixed position for 20 further trials, is certainly a reference-memory procedure. In the absence of separate measures of working memory in this experiment, we cannot comment further on the adequacy of that hypothesis. However, we suspect that claims about the integrity of spatial reference memory after more restricted fimbria-fornix lesions and extensive preoperative training[20] may provide a misleading picture f normal hippocampal function.

The present results show that normal rats can navigate in an appropriate direction towards a hidden object; they do not merely recognize a place when they reach it. Whether this type of learning involves or is different from conventional associative learning deserves further scrutiny. But given that place units detected so far in the hippocampus[1-4] respond only with respect to places in which the rat is presently situated as opposed to places to which it intends to go, these results pose a challenge for electrophysiologists attempting to explain the neural mechanisms by which the hippocampus processes spatial information.

We thank A. Ainsworth for advice about histology, C. Barman for his assistance in constructing the apparatus, and numerous colleagues for their comments on earlier drafts of the paper. This research was supported in part by MRC research grant G979/265/N.

Received 18 January; accepted 14 April 1982.

1. O'Keefe, J. *Brain Res.* **34**, 171–175 (1971).
2. Best, P. J. & Ranck, J. B. Jr *Soc. Neurosci. Abstr.* **1**, 538 (1975).
3. O'Keefe, J. *Expl Neurol.* **51**, 78–109 (1975).
4. Olton, D. S., Branch, M. & Best, P. J. *Expl Neurol.* **58**, 387–409 (1978).
5. O'Keefe, J., Nadel, L., Keigthly, S & Kill, D. *Expl Neurol.* **58**, 387–409 (1978).
6. Olton, D. S., Walker, J. A. & Gage, F. A. *Brain Res.* **139**, 295–308 (1978).
7. Winson, J. *Science* **201**, 160–163 (1978).
8. Barnes, C. A. *J. comp. physiol. Psychol.* **93**, 74–104 (1979).
9. Morris, R. G. M. *Learn Motiv.* **12**, 239–260 (1981).
10. Cowey, A. in *Discrimination* (ed. Weiskrantz, L.) 189–238 (Cambridge University Press, 1968).
11. Milner, A. D., Goodale, M. A. & Morton, M. *J. comp. physiol. Psychol.* **93**, 1015–1023 (1979).
12. Rawlins, J. N. P. & Bennett, J. *Physiol. Behav.* **24**, 415–416 (1980).
13. Rawlins, J. N. P., Feldon, J. & Gray, J. A. *Expl Brain. Res.* **38**, 49–63 (1980).
14. Schapiro, S., Salas, M. & Vukovich, K. *Science*, **168**, 147–149 (1970).
15. Gaflan, D. *J. comp. physiol Psychol.* **86**, 1100–1109 (1974).
16. Mishkin, M. *Nature* **273**, 297–298 (1978).
17. Olton, D. S., Becker, J. T. & Handelmann, G. E. *Behav. Brain Sci.* **2**, 313–365 (1979).
18. Gray, J. A. *The Neuropsychology of Anxiety* (Oxford University Press, in the press).
19. Weiskrantz, L. *Proc. R. Soc.* B (in the press).
20. Olton, D. S. & Papas, B. *Neuropsychologia* **17**, 669–682 (1979).

Evidence for dendritic competition in the developing retina

V. H. Perry & R. Linden

Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, UK

At present little is known of the rules regulating dendritic morphology. Several studies have demonstrated that the shape of the dendritic tree depends on its afferent supply[1,2]. The ganglion cells of the retina provide a particularly useful cell type for the study of neurone development as they develop independently of afferents from other brain regions. If the ganglion cells alone are destroyed in a small patch of the developing retina, it is possible to examine how the absence of neighbouring neurones of the same type influences the development of the ganglion cells around the depleted area. The development of the normal laminar pattern of the retina is not disturbed by the loss of these cells[3]. We show here that the dendrites of ganglion cells around the depleted area are preferentially directed towards this region. The orientation of ganglion cell dendrites is strongly influenced by neighbouring cells and we suggest that during normal development, dendrites compete for their afferents.

Experiments were performed on 11 hooded Lister rats. On the day of birth, the rats were anaesthetized by hypothermia and a small lesion was made in the temporal retina of one eye using a fine 28-gauge needle passed through the sclera approximately half-way between the optic disk and the limbus. After 2–3 months, the animal were anaesthetized with an intraperitoneal injection of 3.0 ml per kg of chlor-nembutal (2.1 g of chloral hydrate + 0.5 g of sodium pentobarbital in 50 ml of 0.9% saline). A series of six injections of 0.15–0.25 μl of horseradish peroxidase (HRP; Boehringer) (50% w/v in 2% dimethyl sulphoxide) were made stereotaxically into the optic tract, using a 1-μl Hamilton syringe. The animals were killed painlessly after 24 h, perfused with 0.9% saline and the eyes removed. The retinae were prepared as whole mounts[4], and reacted in a modified Hanker–Yates solution[5]. After washing in 0.1 M phosphate buffer (*p*H 7.2) for several hours, each retina was transferred to 50 ml solution of 0.1 M sodium

0028-0836/82/250683—03$01.00

Memory consolidation and the amygdala: a systems perspective

James L. McGaugh

The basolateral region of the amygdala (BLA) plays a crucial role in making significant experiences memorable. There is extensive evidence that stress hormones and other neuromodulatory systems activated by arousing training experiences converge in regulating noradrenaline-receptor activity within the BLA. Such activation of the BLA modulates memory consolidation via BLA projections to many brain regions involved in consolidating lasting memory, including the hippocampus, caudate nucleus, nucleus basalis and cortex. Investigation of the involvement of BLA projections to other brain regions is essential for understanding influences of the amygdala on different aspects and forms of memory.

Published online: 18 July 2002

> '...the amygdala (acts) directly on cortical neurons to alter...their responsiveness to the discrete impulses that reach the cortex...these deep nuclei could easily modify the ease and completeness of experience fixation even if the nuclei were not themselves the loci of engrams.' – Ralph Gerard 1961 [1]

The hypothesis that the amygdala is involved in the consolidation of newly created memories is, as the quotation above indicates, not a new idea. But it was clearly a prescient one. Although findings reported more than six decades ago suggested that the amygdala might play a role in learning and memory [2], the amygdala did not figure either prominently or consistently as a brain region important for memory in the ensuing decades. The area was for many years the Cinderella of memory research – in the background and rarely noticed. Lashley's pioneering investigations of cortical function in memory (or lack thereof) [3] dominated research examining brain systems involved in learning at that time. The research focus shifted abruptly with the publication of Scoville and Milner's report of the effects of bilateral surgical removal of the medial temporal lobes, including the anterior hippocampus and the amygdala [4]. The finding that the patient H.M. had, and has to this day [5], severe anterograde amnesia for explicit or declarative memory drew the focus of research attention to the hippocampus [6,7]. The fact that H.M. also lost his amygdala (bilaterally) mostly escaped attention.

James L. McGaugh
Center for the Neurobiology of Learning and Memory and Dept of Neurobiology and Behavior, University of California, Irvine, CA 92697-3800, USA.

For several decades, the intriguing findings of research on the hippocampus strongly overshadowed interest in the possible involvement of the amygdala in learning and memory. A few studies, including those of Weiskrantz [8] and Jones and Mishkin [9] (which suggested that the amygdala might play a role in enabling the learning of associations between cues and reinforcements), kept the interest from fading completely. Even Goddard's seminal finding that electrical stimulation of the amygdala produced retrograde amnesia [10] failed to attract significant attention. Gerard's suggestion that the amygdala might directly influence memory consolidation in cortical neurons [1] also failed to stimulate inquiry. In those decades, a word-association test using the word 'amygdala' would certainly not have produced the short-latency response 'memory'.

Research findings of the past couple of decades have now focused a strong spotlight on the amygdala. Cinderella has been invited to the brain-systems and memory ball, and the amygdala has joined the select list of brain structures thought to be involved in learning and memory. Importantly, the findings suggest that the nuclei of the amygdala could have several roles in learning and memory, including attention [11], cue-, place- and object-reward associations [11–15], conditioned taste aversion [16], appetitive conditioning and drug addiction [17], and conditioned fear and anxiety [18,19]. Moreover, extensive evidence now strongly supports Gerard's long-ignored suggestion [1] that the amygdala is involved in modulating memory consolidation [20,21].

Much current research is guided by the hypothesis that the amygdala, especially the basolateral complex of the amygdala (BLA), could be a locus of neuroplasticity underlying the consolidation of Pavlovian fear conditioning [18,19,22]. This hypothesis suggests that understanding the cellular mechanisms underlying long-term potentiation (LTP) within the amygdala might reveal how fear-based memory is formed and stored within the amygdala [23,24]. However, it is important to note that it is not yet clear how, or whether, this hypothesis can accommodate the extensive findings that complete lesions of the BLA do not prevent fear-based learning – including Pavlovian fear conditioning [25–28]. Additionally, such lesions do not prevent Pavlovian appetitive conditioning or other types of appetitively based learning [11]. Thus, it is currently premature to draw any firm conclusion concerning the hypothesis that the BLA could be a locus of neuroplasticity underlying memory for any kind of training. However, it is not premature to conclude that there is strong support for Gerard's suggestion that the amygdala has an important role in regulating memory consolidation at other brain loci, even if it is not the locus of engrams [1].

http://tins.trends.com 0166-2236/02/$ – see front matter PII: S0166-2236(02)02211-7

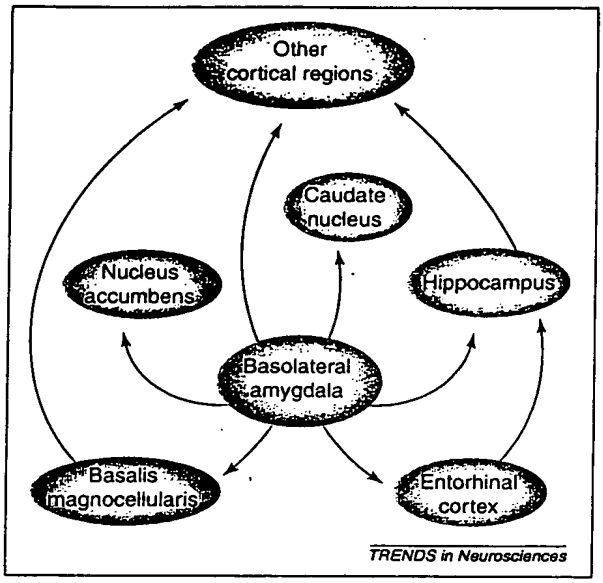


Fig. 1. Projections from the basolateral complex of the amygdala to other brain areas involved in memory consolidation.

Modulation of memory consolidation by the amygdala

There is considerable evidence that drugs and neurotransmitters infused into the amygdala modulate memory consolidation. Gallagher and colleagues [29,30] were the first to report that drugs infused into the amygdala influence consolidation of memory for inhibitory avoidance training. Administration of β-adrenaline-receptor antagonists into the amygdala impaired 24 h retention when administered immediately after training but had no effect when administered 6 h after training. Intra-amygdala infusions of noradrenaline, when administered together with the β-adrenaline-receptor antagonists, attenuated the memory impairment. Importantly, many subsequent studies found that noradrenaline produces dose-dependent and time-dependent enhancement of memory consolidation when infused into the amygdala shortly after inhibitory avoidance training, or training on several other kinds of tasks [31–34].

Post-training peripheral infusions of adrenaline, as well as either peripheral or intra-amygdala administration of drugs affecting the GABA-, opioid-, glucocorticoid- or muscarinic ACh-receptor systems in the amygdala, also have dose- and time-dependent influences on memory consolidation [15,30,35–45]. These effects, with the exception of the influences of ACh, involve modulation of noradrenaline activation within the amygdala [30,31,46–48]. Moreover, the BLA is the crucial region of the amygdala for mediating these neuromodulatory influences on memory consolidation [43–45,49,50]. Drug infusions administered selectively into the immediately adjacent central amygdala do not affect memory consolidation [38,47,51]. Furthermore, selective lesions of the BLA (but not the central nucleus) block the memory-modulating effects of peripherally administered drugs and hormones [20,52–54].

M dulation f memory c nsolidati n by the BLA: th role f eff rent projecti ns from the amygdala

The BLA does not work alone in performing its role in modulating memory consolidation. The BLA projects to many brain regions, including various cortical regions, the hippocampus, basal forebrain, the nucleus accumbens (NAc) and the striatum [55–57] (Fig. 1). There is now considerable evidence that thes projections are crucial in mediating BLA influences on the consolidation of different forms of memory.

Lesions of the stria terminalis (ST), a major pathway connecting the amygdala to other brain regions (including the NAc and dorsal striatum), block the effects of electrical stimulation of the amygdala on memory for inhibitory avoidance training – but do not otherwise impair acquisition or retention [58]. Lesions of the ST also block the memory-enhancing effects of noradrenaline infused into the amygdala after training [32] as well as the memory-modulating effects of adrenaline and those of drugs affecting the opiate, glucocorticoid and muscarinic-ACh systems [46,58–61]. Findings of several studies indicate that the BLA projections to the NAc via the ST are crucial for mediating BLA influences on memory consolidation. As was found with ST lesions, NAc lesions block the memory-enhancing effects of the synthetic glucocorticoid dexamethasone when this is administered systemically after inhibitory avoidance training. Furthermore, combination of a unilateral NAc lesion with a contralateral BLA lesion also blocks dexamethasone-induced memory enhancement [62].

Modulation of memory consolidation by the amygdala: the roles of the caudate nucleus and hippocampus

The BLA–ST pathway provides a major efferent projection enabling BLA influences on other brain regions involved in memory consolidation. It is well established that injection of drugs affecting ACh receptors into the striatum influences the consolidation of memory for inhibitory avoidance training [63,64]. The amygdala–ST–striatum connection is crucial for this influence, as ST lesions block the memory enhancement that is induced by infusions of the muscarinic ACh-receptor agonist oxotremorine directly into the striatum immediately after training [65]. There is also extensive evidence that the amygdala influences hippocampal memory consolidation processes. Packard and Chen [66] reported that infusions of glutamate administered into the hippocampus after training on a food-rewarded maze task enhanced memory consolidation and that concurrent inactivation of the amygdala with lidocaine blocked the enhancement.

Lesions of the ST or of the BLA also block the memory-enhancing effects of systemically administered dexamethasone [52,60]. As glucocorticoic receptors are densely located in the hippocampus, the hippocampus is one likely locus of the glucocorticoid influence on memory consolidation. Lesions of the BLA

or NAc block the memory enhancement that is induced by infusions of a glucocorticoid-receptor agonist directly into the hippocampus after inhibitory avoidance training [61,67]. These findings strongly suggest that BLA–ST–NAc projections are an important pathway mediating BLA influences on memory consolidation involving the hippocampus. As discussed above, activation of noradrenaline receptors within the BLA appears to be essential for the amygdala to influence memory consolidation. The finding that infusions of β-adrenaline-receptor antagonists into the BLA also block the memory-enhancing effects of a glucocorticoid-receptor agonist administered into the hippocampus after training [68] provides further evidence of the role of noradrenaline in the BLA – as well as of the influence of the BLA on hippocampal function in memory consolidation. The report that lesions of the amygdala block the impairing effects of acute stress on hippocampal LTP and water-maze spatial learning [69] provides additional evidence of amygdala–hippocampus interactions in memory formation [70].

Many studies have reported findings suggesting that the caudate nucleus and hippocampus are involved in consolidating different forms or types of memory. Several 'double dissociation' studies [71–74] have reported that drug treatments and lesions affecting the caudate nucleus selectively influence cued learning and memory, whereas the same treatments affecting the hippocampus selectively influence spatial and relational learning. Thus, the caudate nucleus and hippocampus appear to be dedicated to the consolidation of different kinds of information or forms of memory. By contrast, there is evidence that the amygdala is promiscuous in its influence on the consolidation of different forms of memory. Amphetamine infused into the amygdala after training enhances both cued and spatial/relational memory [75,76]. Furthermore, infusions of lidocaine into the caudate nucleus after training prevent the amygdala-induced enhancement of memory for cued water-maze training, whereas lidocaine infused into the hippocampus after training prevents the amygdala-induced enhancement of memory for spatial water-maze training [76]. These findings indicate that the amygdala modulates the consolidation of memory for cued training by influencing the caudate nucleus, and the consolidation of spatial and relational memory by influencing the hippocampus.

Modulation of memory consolidation and hippocampal LTP by the BLA

Other recent findings provide further evidence consistent with the hypothesis that the amygdala provides a modulatory and time-limited influence on hippocampal functioning in memory consolidation [77]. Infusions of the protein kinase II (CaMKII) inhibitor KN-62 induce retrograde amnesia when administered into the amygdala or CA1 region of the hippocampus immediately after inhibitory avoidance training. Infusions of drugs that stimulate protein kinase A (PKA) (e.g. noradrenaline and the cAMP analog 8-Br-cAMP) enhance memory when administered into the hippocampus, but not the amygdala, 3 h after training. As noradrenaline or 8-Br-cAMP infused into the hippocampus 3 h after training also attenuates the amnesia induced by KN-62 administered into the amygdala immediately after training, the amygdala-induced amnesia appears to reflect disruption of modulatory influences rather than disruption of memory consolidation within the amygdala. By contrast, the hippocampus does appear to be crucial for memory consolidation. Noradrenaline or 8-Br-cAMP infused into the hippocampus 3 h after training does not attenuate amnesia induced by KN-62 infused into the hippocampus immediately after training [78].

Activation of CaMKII and PKA cascades in the hippocampus appears to be crucial for LTP [79] as well as for memory consolidation [80,81]. Many recent studies have reported that the BLA modulates LTP in the hippocampus *in vivo*. Lesions of the BLA or administration of β-adrenaline-receptor antagonists into the BLA block the induction of LTP in the dentate gyrus [82–84]. Moreover, stimulation of the BLA either before or within 30 min following LTP induction enhances LTP [85–87]. It remains to be determined, of course, whether such LTP is causally linked to memory [88].

Influences of BLA–cortical connections on memory consolidation

It is now well established that the cortex is also a crucial locus of memory consolidation. Functional inactivation of cortical regions after training with the GABA-receptor agonist muscimol, with the Na^{2+}-channel blocker tetrodotoxin or by infusion of drugs affecting the cAMP–PKA signaling pathway produces retrograde amnesia for training in several kinds of learning tasks [89–92]. Drug infusions administered into cortical regions can also enhance memory consolidation [90]. Additionally, and importantly, evidence from several recent studies indicates that the BLA modulates cortical functioning in memory consolidation. All nuclei within the BLA complex project directly to the entorhinal cortex [57,93] and firing of BLA neurons activates neurons in the entorhinal cortex [94,95]. As is shown in Fig. 2a, infusions of 8-Br-cAMP into the entorhinal cortex immediately after inhibitory avoidance training enhance retention. Projections from the BLA are essential in enabling the modulation of memory consolidation mediated by the entorhinal cortex, as lesions of the BLA block the memory enhancement [96].

The nucleus basalis magnocellularis (NBM) provides cholinergic projections to the cortex. The finding that functional inactivation of the NBM with infusions of lidocaine impairs the acquisition of conditioned taste aversion indicates that ACh-mediated activation of the cortex is crucial for

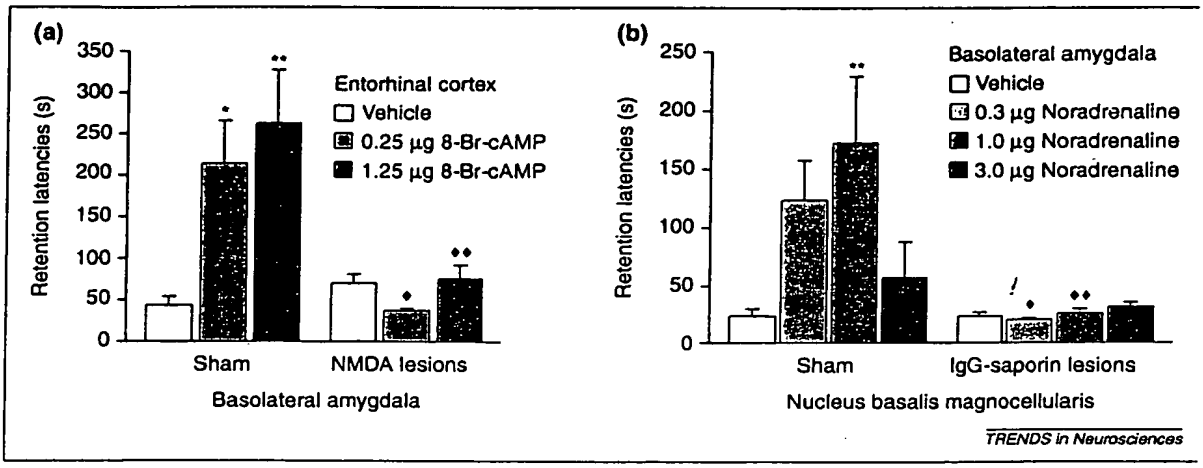


Fig. 2. Interactions of the basolateral complex of the amygdala (BLA) with the cortex in memory consolidation. (a) Lesions of the BLA block the memory-enhancing effects (in a 48 h retention test) of 8-Br-cAMP infused into the entorhinal cortex immediately after training on a one-trial inhibitory avoidance task. $^{*}P<0.05$ and $^{**}P<0.01$ compared to the corresponding control group [96]. $^{\bullet}P<0.05$ and $^{\bullet\bullet}P<0.01$ compared to corresponding 8-Br-cAMP sham-lesion control groups. (b) Lesions of nucleus basalis magnocellularis (NBM) induced by infusions of 192 IgG-saporin block the memory enhancement (in a 48 h retention test), as induced by post-training infusions of noradrenaline administered into the basolateral amygdala immediately after inhibitory avoidance training. $^{*}P<0.01$ and $^{**}P<0.001$ compared to vehicle sham-lesion control groups. $^{\bullet}P<0.05$ and $^{\bullet\bullet}P<0.001$ compared to corresponding sham-lesion animals receiving the same noradrenaline dose [34].

this form of learning – a form that is also known to involve the BLA [97]. Activation of the NBM and the consequent release of ACh in the auditory cortex are essential for the consolidation of changes in cortical function (receptive field plasticity) induced by Pavlovian conditioning using tone-shock pairing [98–101]. The BLA is a principal source of afferents to the NBM [102]. Several lines of evidence suggest that the BLA modulates cortical activity via projections to the NBM mediated largely by the ST. Dringenberg and Vanderwolf [103] reported that BLA stimulation activates cortical EEG activity (i.e. induces low voltage high frequency activity) and that lidocaine infused into the NBM blocks the BLA-induced activation. Moreover, BLA stimulation potentiates the cortical EEG activation evoked by somatosensory stimulation [104]. These findings suggest that BLA activation of the NBM and consequent ACh-mediated activation of the cortex could be important, and possibly essential, for modulation of memory consolidation. This issue was investigated in a recent study of BLA influences on memory in rats with lesions of the NBM induced by 192 IgG-saporin, a p75 nerve growth factor receptor-mediated neurotoxin that selectively destroys cholinergic projections to the cortex [42]. As is shown in Fig. 2b, the dose-dependent enhancement of memory consolidation induced by noradrenaline administered into the BLA after training was completely blocked in animals with 192 IgG-saporin lesions [34]. These findings provide strong support for the hypothesis that BLA–NBM–cortical cholinergic projections play an important, if not crucial, role in mediating the memory-modulating influence of adrenaline-receptor activation of the BLA.

BLA: connections and consequences

The BLA makes good use of its many connections with other brain regions in regulating memory consolidation. Some of the brain regions influenced by the BLA (e.g. the hippocampus and possibly the entorhinal cortex) could regulate the consolidation of long-lasting memory in circuits elsewhere in the brain, whereas others (e.g. the caudate nucleus and NBM-activated cortical regions) might serve as the loci of lasting memories. Learning-induced neuroplasticity within the amygdala [23,24] could play a role in enabling the prolonged post-training modulatory influences of the BLA on memory consolidation in other brain regions.

Considerable evidence now strongly supports the suggestion that the amygdala could influence, '...cortical neurons to...modify the ease and completeness of experience fixation even if the nuclei (are) not themselves the loci of engrams' [1]. To update this hypothesis, we need to add that it is the basolateral complex of the amygdala that serves this important role, and that the BLA acts via projections to brain regions involved in forming and consolidating memories of different kinds of experiences.

Acknowledgements
I thank Larry Cahill, Ivan Izquierdo, Richard Morris, Benno Roozendaal, Georg Striedter and Norman Weinberger for their thoughtful and helpful comments on a previous draft of this paper, and Ann Power and Nancy Collett for assistance in preparing the manuscript.

References

1 Gerard, R.W. (1961) The fixation of experience. In *Brain Mechanisms and Learning* (Fessard, A. *et al.*, eds.) pp. 21–35 Chars C. Thomas
2 Klüver, H. and Bucy, P.C. (1937) 'Psychic blindness' and other symptoms following bilateral temporal lobectomy in rhesus monkeys. *Am. J. Physiol.* 119, 352–353
3 Lashley, K.S. (1950) In search of the engram. *Symp. Soc. Exp. Biol.* 4, 454–482
4 Scoville, W.B. and Milner, B. (1957) Loss of recent memory after bilateral hippocampal lesions. *J. Neurol. Neurosurg. Psychiatry* 20, 11–21
5 Corkin, S. (2002) What's new with the amnesic patient H.M.? *Nat. Rev. Neurosci.* 3, 153–160
6 Squire, L.R. and Kandel, E.R. (1999) *Memory from Mind to Molecules*, Scientific American Library, New York
7 Eichenbaum, H. and Cohen, N.J. (2001) *From Conditioning to Conscious Recollection: Memory Systems of the Brain*, Oxford University Press
8 Weiskrantz, L. (1956) Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *J. Comp. Physiol. Psychol.* 49, 381–391
9 Jones, B. and Mishkin, M. (1972) Limbic lesions and the problem of stimulus-reinforcement associations. *Exp. Neurol.* 36, 362–377

10 Goddard, G.V. (1964) Amygdaloid stimulation and learning in the rat. *J. Comp. Physiol. Psychol.* 58, 23–30
11 Holland, P.C. and Gallagher, M. (1999) Amygdala circuitry in attentional and representational processes. *Trends Cognit. Sci.* 3, 65–73
12 White, N.M. and McDonald, R.J. (2002) Multiple parallel memory systems in the brain of the rat. *Neurobiol. Learn. Mem.* 77, 125–184
13 Easton, A. and Gaffan, D. (2000) The amygdala and the memory of reward: the importance of fibres of passage from the basal forebrain. In *The Amygdala* (Aggleton, J.P., ed.), pp. 569–586, Oxford University Press
14 Baxter, M.G. and Murray, E.A. (2000) Reinterpreting the behavioral effects of amygdala in non-human primates. In *The Amygdala* (Aggleton, J.P., ed.) pp. 545–568, Oxford University Press
15 Salinas, J. *et al.* (1997) Posttraining intra-amygdala infusion of oxotremorine and propranolol modulate storage of memory for reduction in reward magnitude. *Neurobiol. Learn. Mem.* 68, 51–59
16 Lamprecht, R. and Dudai, Y. (2000) The amygdala in conditioned taste aversion: it's there, but where? In *The Amygdala* (Aggleton, J.P., ed.), pp. 331–352, Oxford University Press
17 Everitt, B.J. *et al.* (2000) Differential involvement of the amygdala subsystems in appetitive conditioning and drug addiction. In *The Amygdala* (Aggleton, J.P., ed.), pp. 353–390, Oxford University Press
18 LeDoux, J. (2000) The amygdala and emotion: a view through fear. In *The Amygdala* (Aggleton, J.P., ed.), pp. 289–310, Oxford University Press
19 Davis, M. (2000) The role of the amygdala in conditioned and unconditioned fear and anxiety. In *The Amygdala* (Aggleton, J.P., ed.), pp. 213–288, Oxford University Press
20 McGaugh, J.L. (2000) Memory: A century of consolidation. *Science* 287, 248–251
21 McGaugh, J.L. *et al.* (2000) Amygdala: Role in modulation of memory storage. In *The Amygdala* (Aggleton, J.P., ed.), pp. 391–423, Oxford University Press
22 Maren, S. (1999) Long-term potentiation in the amygdala: a mechanism for emotional learning and memory. *Trends Neurosci.* 22, 561–567
23 Schafe, G.E. *et al.* (2001) Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. *Trends Neurosci.* 24, 540–546
24 Medina, J.F. *et al.* (2002) Parallels between cerebellum- and amygdala-dependent conditioning. *Nat. Rev. Neurosci.* 3, 122–131
25 Cahill, L. *et al.* (2000) The basolateral amygdala complex is involved with, but is not necessary for, rapid acquisition of Pavlovian 'fear' conditioning. *Eur. J. Neurosci.* 12, 3044–3050
26 Killcross, S. *et al.* (1997) Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. *Nature* 388, 377–380
27 Vazdarjanova, A. and McGaugh, J.L. (1998) Basolateral amygdala is not a critical locus for memory of contextual fear conditioning. *Proc. Natl. Acad. Sci. U. S. A.* 95, 15003–15007
28 Lehmann, H. *et al.* (2000) Amygdala lesions do not impair shock-probe avoidance retention performance. *Behav. Neurosci.* 114, 107–116
29 Gallagher, M. *et al.* (1977) Memory formation: evidence for a specific neurochemical system in the amygdala. *Science* 198, 423–425
30 Gallagher, M. *et al.* (1981) A neuropharmacology of amygdaloid systems which contribute to learning and memory. In *The Amygdaloid Complex* (Y. Ben-Ari, ed.), pp. 343–354, Elsevier
31 Liang, K.C. *et al.* (1986) Modulating effects of posttraining epinephrine on memory: involvement of the amygdala noradrenergic system. *Brain Res.* 368, 125–133
32 Liang, K.C. *et al.* (1990) Involvement of amygdala pathways in the influence of posttraining amygdala norepinephrine and peripheral epinephrine on memory storage. *Brain Res.* 508, 225–233
33 Hatfield, T. and McGaugh, J.L. (1999) Norepinephrine infused into the basolateral amygdala posttraining enhances retention in a spatial water maze task. *Neurobiol. Learn. Mem.* 71, 232–239
34 Power, A.E. *et al.* (2002) Lesions of the nucleus basalis magnocellularis induced by 192 IgG-saporin block memory enhancement with posttraining norepinephrine in the basolateral amygdala. *Proc. Natl. Acad. Sci. U. S. A.* 99, 2315–2319
35 Brioni, J.D. *et al.* (1989) Involvement of the amygdala GABAergic system in the modulation of memory storage. *Brain Res.* 487, 105–112
36 Izquierdo, I. *et al.* (1990) Post-training down-regulation by a GABA-A mechanism in the amygdala modulated by endogenous benzodiazepines. *Behav. Neural Biol.* 54, 105–109
37 Izquierdo, I. *et al.* (1992) Neurotransmitter receptors involved in memory processing by the amygdala, medial septum and hippocampus of rats. *Behav. Neural Biol.* 58, 16–25
38 Roozendaal, B. and McGaugh, J.L. (1997a) Glucocorticoid receptor agonist and antagonist administration into the basolateral but not central amygdala modulates memory storage. *Neurobiol. Learn. Mem.* 67, 176–179
39 Wilensky, A.E. *et al.* (2000) The amygdala modulates memory consolidation of fear-motivated inhibitory avoidance learning but not classical conditioning. *J. Neurosci.* 20, 7059–7066
40 Dalmaz, C. *et al.* (1993) Noradrenergic and cholinergic interactions in the amygdala and the modulation of memory storage. *Behav. Brain Res.* 58, 167–174
41 Introini-Collison, I.B. *et al.* (1996) Amygdala β-noradrenergic influences on memory storage involve cholinergic activation. *Neurobiol. Learn. Mem.* 65, 57–64
42 Power, A.E. *et al.* (2000) Glucocorticoid enhancement of memory consolidation in the rat is blocked by muscarinic receptor antagonism in the basolateral amygdala. *Euro. J. Neurosci.* 12, 3481–3487
43 Vazdarjanova, A. and McGaugh, J.L. (1999) Basolateral amygdala is involved in modulating consolidation of memory for classical fear conditioning. *J. Neurosci.* 19, 6615–6622
44 Ferry, B. *et al.* (1999) Involvement of α_1-adrenergic receptors in the basolateral amygdala in modulation of memory storage. *Eur. J. Pharmacol.* 372, 9–16
45 Ferry, B. *et al.* (1999) Basolateral amygdala noradrenergic influences on memory storage are mediated by an interaction between beta- and $alpha_1$-receptors. *J. Neurosci.* 19, 5119–5123
46 Introini-Collison, I.B. *et al.* (1989) Memory-enhancement with intra-amygdala posttraining naloxone is blocked by concurrent administration of propranolol. *Brain Res.* 476, 94–101
47 Quirarte, G.L. *et al.* (1997) Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. *Proc. Natl. Acad. Sci. U. S. A.* 94, 14048–14053
48 Roozendaal, B. *et al.* (2002) Glucocorticoids interact with the basolateral amygdala β-adrenoceptor-cAMP/PKA system in influencing memory consolidation. *Eur. J. Neurosci.* 15, 553–560
49 Hatfield, T. *et al.* (1999) Response of amygdalar norepinephrine to footshock and GABAergic drugs using *in vivo* microdialysis and HPLC. *Brain Res.* 835, 340–345
50 Ferry, B. and McGaugh, J.L. (1999) Clenbuterol administration into the basolateral amygdala post-training enhances retention in an inhibitory avoidance task. *Neurobiol. Learn. Mem.* 72, 8–12
51 DaCunha, C. *et al.* (1999) Microinfusions of flumazenil into the basolateral but not the central nucleus of the amygdala enhance memory consolidation in rats. *Neurobiol. Learn. Mem.* 72, 1–7
52 Roozendaal, B. and McGaugh, J.L. (1996) Amygdaloid nuclei lesions differentially affect glucocorticoid-induced memory enhancement in an inhibitory avoidance task. *Neurobiol. Learn. Mem.* 65, 1–8
53 Roozendaal, B. *et al.* (1996) Basolateral amygdala lesions block glucocorticoid-induced modulation of memory for spatial learning. *Behav. Neurosci.* 110, 1074–1083
54 McGaugh, J.L. and Roozendaal, B. (2002) Role of adrenal stress hormones in forming lasting memories in the brain. *Curr. Opin. Neurobiol.* 12, 205–210
55 Young, M.P. (1993) The organization of neural systems on the primate cerebral cortex. *Proc. R. Soc. Lond. B Biol. Sci.* 252, 13–18
56 Pitkänen, A. (2000) Connectivity of the rat amygdaloid complex. In *The Amygdala* (Aggleton, J.P., ed.), pp. 31–117, Oxford University Press
57 Petrovich, G.D. *et al.* (2001) Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. *Brain Res. Rev.* 38, 247–289
58 Liang, K.C. and McGaugh, J.L. (1983) Lesions of the stria terminalis attenuate the amnestic effect of amygdaloid stimulation on avoidance responses. *Brain Res.* 274, 309–318
59 McGaugh, J.L. *et al.* (1986) Stria terminalis lesions attenuate the effects of posttraining naloxone and β-endorphin on retention. *Behav. Neurosci.* 100, 839–844
60 Roozendaal, B. and McGaugh, J.L. (1996) The memory-modulatory effects of glucocorticoids depend on an intact stria terminalis. *Brain Res.* 709, 243–250
61 Roozendaal, B. *et al.* (2001) Basolateral amygdala-nucleus interactions in mediating glucocorticoid effects on memory consolidation. *J. Neurosci.* 21, 2518–2525
62 Setlow, B. *et al.* (2000) Involvement of a basolateral amygdala complex – nucleus accumbens pathway in glucocorticoid-induced modulation of memory storage. *Eur. J. Neurosci.* 12, 367–375
63 Prado-Alcalá, R.A. and Cobos-Zapiaín, G.G. (1979) Improvement of learned behavior through cholinergic stimulation of the caudate nucleus. *Neurosci. Lett.* 14, 253–258

64 Prado-Alcalá, R.A. (1995) Serial and parallel processing during memory consolidation. In *Plasticity in the Central Nervous System: Learning and Memory*. (McGaugh, J.L. *et al.*, eds) pp. 57–65, Lawrence Erlbaum
65 Packard, M.G. *et al.* (1996) Stria terminalis lesions attenuate memory enhancement produced by intra-caudate nucleus injections of oxotremorine. *Neurobiol. Learn. Mem.* 65, 278–282
66 Packard, M.G. and Chen, S.A. (1999) The basolateral amygdala is a cofactor in memory enhancement produced by intrahippocampal glutamate injections. *Psychobiology* 27, 377–385
67 Roozendaal, B. and McGaugh, J.L. (1997) Basolateral amygdala lesions block the memory-enhancing effect of glucocorticoid administration in the dorsal hippocampus of rats. *Eur. J. Neurosci.* 9, 76–83
68 Roozendaal, B. *et al.* (1999) Basolateral amygdala noradrenergic influence enables enhancement of memory consolidation induced by hippocampal glucocorticoid receptor activation. *Proc. Natl. Acad. Sci. U. S. A.* 96, 11642–11647
69 Morris, R.G.M. (1984) Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods* 11, 47–60
70 Kim, J.J. *et al.* (2001) Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning. *J. Neurosci.* 21, 5222–5228
71 Packard, M.G. *et al.* (1989) Differential effects of fornix and caudate lesions on two radial maze tasks: evidence for multiple memory systems. *J. Neurosci.* 9, 1465–1472
72 Packard, M.G. and White, N.M. (1991) Dissociation of hippocampal and caudate nucleus memory systems by post-training intracerebral injection of dopamine agonists. *Behav. Neurosci.* 105, 295–306
73 Packard, M.G. and McGaugh, J.L. (1992) Double dissociation of fornix and caudate nucleus lesions on acquisition of two water maze tasks, further evidence for multiple memory systems. *Behav. Neurosci.* 106, 439–446
74 Packard, M.G. and McGaugh, J.L. (1996) Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol. Learn. Mem.* 65, 65–72
75 Packard, M.G. *et al.* (1994) Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. *Proc. Natl. Acad. Sci. U. S. A.* 91, 8477–8481
76 Packard, M.G. and Teather, L. (1998) Amygdala modulation of multiple memory systems: hippocampus and caudate-putamen. *Neurobiol. Learn. Mem.* 69, 163–203
77 Wolfman, C. *et al.* (1994) Intrahippocampal or intra-amygdala infusion of Kn62, a specific inhibitor of calcium/calmodulin-dependent protein kinase II, causes retrograde amnesia in the rat. *Behav. Neural Biol.* 61, 203–205
78 Barros, D.M. *et al.* (1999) Stimulators of the cAMP cascade reverse amnesia induced by intra-amygdala but not intra-hippocampal KN-62 administration. *Neurobiol. Learn. Mem.* 71, 94–103
79 Malenka, R.C. and Nicoll, R.A. (1999) Long-term potentiation–a decade of progress? *Science* 285, 1870–1874
80 Bernabeu, R. *et al.* (1997) Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. *Proc. Natl. Acad. Sci. U. S. A.* 94, 7041–7046
81 McGaugh, J.L. and Izquierdo, I. (2000) Pharmacology's contribution to research on the mechanisms of memory formation. *Trends Pharmacol. Sci.* 21, 208–210
82 Ikegaya, Y. *et al.* (1994) Attenuated hippocampal long-term potentiation in basolateral amygdala-lesioned rats. *Brain Res.* 656, 157–164
83 Ikegaya, Y. *et al.* (1995) Requirement of basolateral amygdala neuron activity for the induction of long-term potentiation in the dentate gyrus *in vivo*. *Brain Res.* 671, 351–354
84 Ikegaya, Y. *et al.* (1997) Amygdala beta-noradrenergic influence on hippocampal long-term potentiation *in vivo*. *NeuroReport* 8, 3143–3146
85 Ikegaya, Y. *et al.* (1995) High-frequency stimulation of the basolateral amygdala facilitates the induction of long-term potentiation in the dentate gyrus *in vivo*. *Neurosci. Res.* 22, 203–207
86 Akirav, I. and Richter-Levin, G. (1999) Biphasic modulation of hippocampal plasticity by behavioral stress and basolateral amygdala stimulation in the rat. *J. Neurosci.* 19, 10530–10535
87 Frey, S. *et al.* (2001) Reinforcement of early long-term potentiation (early LTP in dentate gyrus by stimulation of the basolateral amygdala: heterosynaptic induction mechanisms of late-LTP. *J. Neurosci.* 21, 3697–3703
88 Martin, S.J. *et al.* (2000) Synaptic plasticity and memory: and evaluation of the hypothesis. *Annu. Rev. Neurosci.* 23, 649–711
89 Izquierdo, I. *et al.* (1997) Sequential role of hippocampus and amygdala, entorhinal cortex and parietal cortex in formation and retrieval of memory for inhibitory avoidance in rats. *Eur. J. Neurosci.* 9, 786–793
90 Ardenghi, P. *et al.* (1997) Late and prolonged post-training memory modulation in entorhinal and parietal cortex by drugs acting on the cAMP/protein kinase A signalling pathway. *Behav. Pharmacol.* 8, 745–751
91 Baldi, E. *et al.* (1999) Effects of combined medial septal area, fimbria-fornix and entorhinal cortex tetrodotoxin inactivations on passive avoidance response consolidation in the rat. *Brain Res.* 821, 503–510
92 Sacchetti, B. *et al.* (1999) Auditory thalamus, dorsal hippocampus, basolateral amygdala, and perirhinal cortex role in the consolidation of conditioned freezing to context and to acoustic conditioned stimulus in the rat. *J. Neurosci.* 19, 9570–9578
93 Pikkarainen, M. *et al.* (1999) Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. *J. Comp. Neurol.* 403, 229–260
94 Paré, D. and Gaudreau, H. (1996) Projection cells and interneurons of the lateral and basolateral amygdala: distinct firing patterns and differential relation to theta and delta rhythms in conscious cats. *J. Neurosci.* 16, 3334–3350
95 Paré, D. *et al.* (1995) Amygdalo-entorhinal relations and their reflection in the hippocampal formation: generation of sharp potentials. *J. Neurosci.* 15, 2482–2503
96 Roesler, R. *et al.* (2002) Basolateral amygdala lesions block the memory-enhancing effect of 8-Br-cAMP infused into the entorhinal cortex of rats after training. *Eur. J. Neurosci.* 15, 905–910
97 Miranda, M.I. and Bermudez-Rattoni, F. (1999) Reversible inactivation of the nucleus basalis magnocellularis induces disruption of cortical acetylcholine release and acquisition, but not retrieval, of aversive memories. *Proc. Natl. Acad. Sci. U. S. A.* 96, 6478–6482
98 Weinberger, N.M. (1995) Retuning the brain by fear conditioning. In *The Cognitive Neurosciences* (Gazzaniga, M.S., ed.), pp. 1071–1089, MIT Press
99 Weinberger, N.M. (1998) Tuning the brain by learning and by stimulation of the nucleus basalis. *Trends Cognit. Sci.* 2, 271–273
100 Miasnikov, A.A. *et al.* (2001) Muscarinic dependence of nucleus basalis induced conditioned receptive field plasticity. *NeuroReport* 12, 1537–1542
101 Galvan, V.V. and Weinberger, N.M. (2002) Long-term consolidation and retention of learning-induced tuning plasticity in the auditory cortex of the guinea pig. *Neurobiol. Learn. Mem.* 77, 78–108
102 Russchen, F.T. *et al.* (1985) The afferent connections of the substantia innominata in the monkey, *Macaca fascicularis*. *J. Comp. Neurol.* 242, 1–27
103 Dringenberg, H. and Vanderwolf, C. (1996) Cholinergic activation of the electrocorticogram: an amygdaloid activating system. *Exp. Brain Res.* 108, 285–296
104 Dringenberg, H. *et al.* (2001) Enhanced frontal cortex activation in rats by convergent amygdaloid and noxious sensory signals. *NeuroReport* 12, 2395–2398